REMARKS

With entry of this amendment, claims 1, 3, 5 and 7-16 are pending in the application. Reconsideration is requested. Claims 1, 3, and 5 have been amended to overcome indefiniteness rejections. New claims 12-16 recite the composition of adjuvants A-E, as described at pages 38-39 of the specification. No new matter has been added.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1, 5 and 7-11 were rejected under 35 U.S.C. §112, first paragraph, as failing to provide an enabling disclosure without evidence that the claimed vaccine and method produce a protective immune response. The Examiner's attention is directed to the Declaration of Dr. Jeffrey Lyon, one of the inventors on the application, filed herewith, that presents challenge data in Aotus monkeys. Animals underwent a series of vaccine injections with a vaccine according to the invention, and were subsequently challenged by exposure to the FVO strain of *P. falciparum*. The vaccine of the invention produced a significant reduction in infection by *P. falciparum*, as shown in Figure 3 of the Declaration.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 1, 3, 5 and 7-11 have been rejected as being indefinite. It is the Examiner's position that the terms adjuvant A, adjuvant B, etc. are indefinite. Claims 1, 3 and 5 have been amended to remove the recitation of specific adjuvants A, B, C, D, and E. Claims 12-16 have been added to recite the use of specific adjuvants, as identified in the specification at pages 38-39. The adjuvants are identified by composition, and are believed to be free of the indefiniteness rejection. Withdrawal of the rejection is accordingly requested.

All objections and rejections having been addressed, it is respectfully requested that the rejections be withdrawn and a Notice of Allowance issued. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is hereby invited to telephone the undersigned at the number provided.

Respectfully submitted,

Date: 4/1/05

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	10/057,532
Applicant	:	LYON et al.
Filed	:	January 25, 2002
Art Unit	:	1645
Examiner	:	P. Baskar
Docket No.	:	38644-197852
Customer No.	:	26694

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION

Sir:

I, Jeffrey A. Lyon, do hereby declare and state as follows:

1. I hold a Ph. D. in Chemistry/Biochemistry from the University of South Carolina, and have worked in the field of Malaria Immunology for more than 20 years I have published more than 30 scholarly papers in the peer reviewed literature on the molecular biology and immunology of erythrocytic stage *Plasmodium falciparum*, and I am named as an inventor on several patents in this field as well.

2. I am an inventor on the above-referenced U.S. patent application, and have read the Office Action issued October 1, 2004 (the OA).

3. In the patent application, a vaccine and an immunogenic composition are disclosed and claimed, comprising *P. falciparum* 3D7 MSP-1_{42} and an adjuvant.

4. In the OA, claims 1, 5, and 7-11 were rejected by the Examiner under 35 USC § 112, first paragraph. The Examiner said that "the specification fails to disclose *(1)* Animals immunized

with claimed immunogenic composition are able to inhibit malaria infection upon challenge either with homologous or heterologous *Plasmodium falciparum*."

5. The following experiments were performed under my direction at WRAIR and demonstrate a protective effect of a vaccine composition comprising a recombinant protein encoded by the MSP-1_{42} gene fragment and an adjuvant comprising QS21 and 3D-MPL in an oil in water emulsion (AS02A).

6. The MSP-1_{42} gene fragment from the 3D7 strain of *Plasmodium falciparum* was expressed as soluble protein in *Escherichia coli* and purified according to Good Manufacturing Practice specifications, and according to the method described in the patent application at pages 52 to 55. This recombinant protein is referred to as FMP1. The GMP protein was reactive with several functional, conformation-dependent monoclonal antibodies raised against *P. falciparum* malaria parasites (Angov, E., B. M. Aufiero, A. M. Turgeon, M. Van Handenhove, C. F. Ockenhouse, K. E. Kester, D. S. Walsh, J. S. McBride, M. C. Dubois, J. Cohen, J. D. Haynes, K. H. Eckels, D. G. Heppner, W. R. Ballou, C. L. Diggs, and J. A. Lyon. 2003. Development and pre-clinical analysis of a *Plasmodium falciparum* merozoite surface protein- (42) malaria vaccine. Mol. Biochem.Parasitol. 128:195–204.)

7. The FMP1 protein was formulated with the adjuvant described above and *Aotus nancymai* (Aotus monkeys) were immunized with this vaccine and challenged with FVO strain *P. falciparum* erythrocytic-stage malaria parasites. The FVO *Plasmodium falciparum* strain is a strain adapted to *Aotus* monkeys. The trial included two groups of monkeys. The monkeys of one group received FMP1 adjuvanted with AS02A, and the monkeys of the other group received AS02A alone. The primary endpoint for the study was cumulative day 11 parasitemia, which is defined as the sum of the daily parasite counts per µl of blood until the day before the first

treatment for malaria infection was given. No homologous challenge was performed because 3D7-like *P. falciparum* parasites do not infect *Aotus nancymai*.

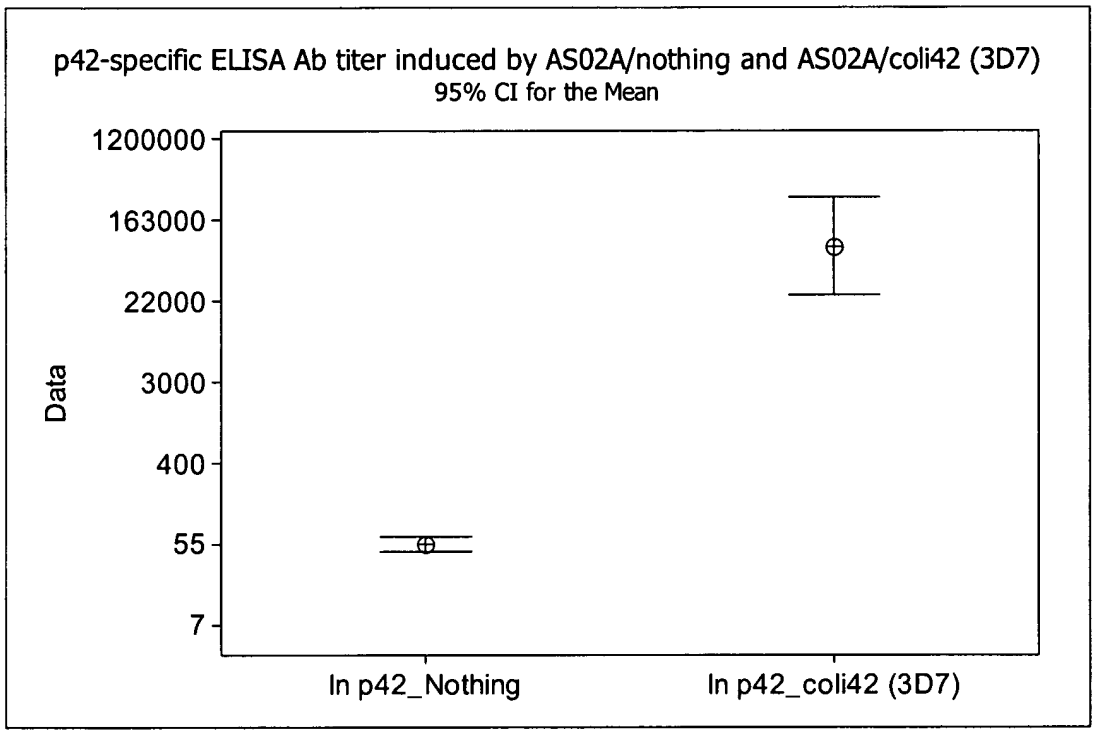
8. *Aotus nancymai* Vaccination: The monkeys used in this study were spleen-intact *Aotus nancymai* of either sex with no known history of *Plasmodium* species infections, as determined by parasitological and serological examinations; veterinary examination revealed that the animals were in good health and free of tuberculosis. The monkeys weighed no less than about 700 grams at the start of the study and were stratified into two groups of six animals according to weight and sex. Groups were then assigned to the vaccine group and control group. Each animal was vaccinated four times with 50 μg of either FMP1 adjuvanted with AS02A or with AS02A alone.

9. Challenge: The *P. falciparum* Vietnam Oak Knoll (FVO) is adapted to karyotype I *Aotus* monkeys and produces a lethal rapid high-density parasitemia in spleen-intact naïve *Aotus* monkeys [cite a reference that supports this]. For challenge, parasitized erythrocytes from a donor *Aotus* monkey were diluted to 20,000 parasitized RBC (PRBC)/ml in sterile RPMI–1640 tissue culture media and 0.5 ml was administered intravenously via a femoral vein.

10. Evaluation: Beginning three days following challenge and for 53 successive days thereafter (a total of 56 days), Giemsa stained blood smears were made to quantify parasites per μl of blood (Earle, W. C., and M. Perez. 1931. Enumeration of parasites in the blood of malarial patients. J. Lab. Clin. Med. 19:1124–1130.) When parasite counts exceeded 80,000/μl of blood, parasite density was quantified from a thin blood smear. Monkeys that developed parasitemia of > 200,000 parasites/μl of blood were treated with curative doses of mefloquine and quinine. Antibody responses to vaccination were measured with serum taken on the day of challenge.

11. Statistical Analysis: Data were transformed to the natural logarithm because individual values were spread over a range that exceeded ten-fold. After transformation, data for the groups were evaluated for equal variance among groups, and tested for group-wise differences using either an independent Student's t-tests (two-sample t-test).

Figure 1. FMP1/AS02A ("ln p42_coli42 (3D7)" in the figure below) induces significantly higher titers of p42-specific Ab than AS02A alone ("ln p42_nothing"). Sera for the analysis were collected on the day of challenge.



Two-Sample T-Test and CI: ln p42_Nothing, ln p42_coli42 (3D7) (Student's t-test)

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Two-sample T for ln p42_Nothing vs ln p42_coli42 (3D7): natural log transformations,
equal variance not assumed

                  N   Mean  StDev  SE Mean
ln p42_Nothing    6  4.020  0.180    0.073
ln p42_coli42 (3  6  11.37   1.15     0.47


Difference = mu (ln p42_Nothing) - mu (ln p42_coli42 (3D7))
Estimate for difference:  -7.35485
95% upper bound for difference:  -6.39636
T-Test of difference = 0 (vs <): T-Value = -15.46  P-Value = 0.000  DF = 5
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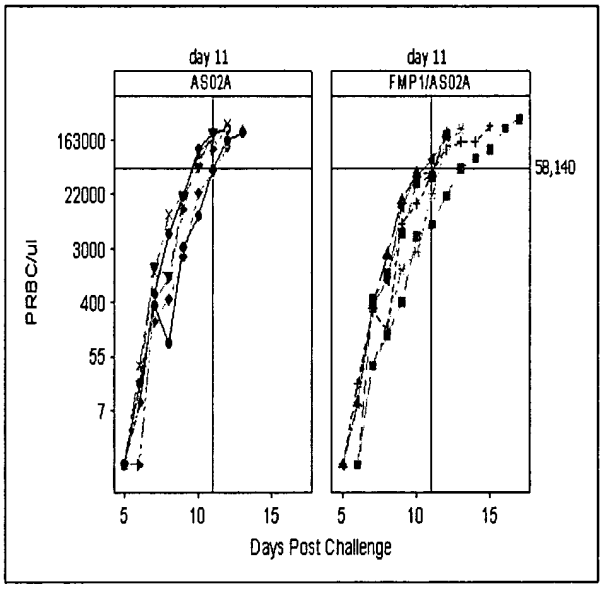
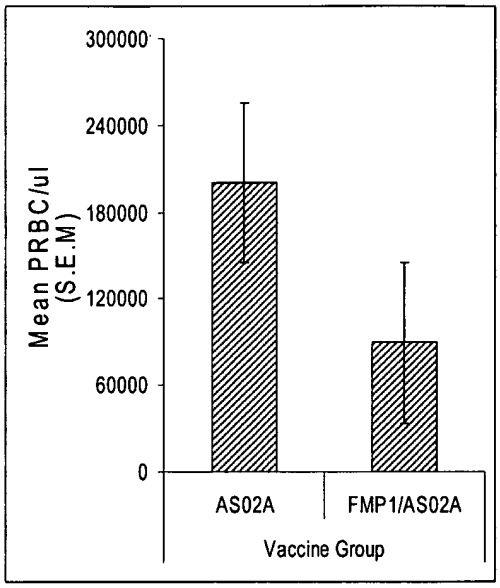
Figure 2. Animals vaccinated with FMP1/AS02A have a significantly lower parasitemia after heterologous *P. falciparum* (FVO strain) challenge compared with animals receiving AS02A alone. A. Daily parasite rates (PRBC/ul). B. Cumulative Day 11 parasitemia (sum of daily parasite rates from 3 day – 11 days post-challenge).

A. Post-Challenge Infection Kinetics



B. Cumulative Day 11 Parasitemia (S.E.M)



Test for Equal Variances: ln(day 11) versus Vaccine/Control

95% Bonferroni confidence intervals for standard deviations

Vaccine/Control	N	Lower	StDev	Upper
FMP1/AS02A	6	0.552527	0.942341	2.69598
AS02A	5	0.410448	0.733137	2.53632

F-Test (normal distribution)
Test statistic = 1.65, p-value = 0.647

Levene's Test (any continuous distribution)
Test statistic = 0.11, p-value = 0.749

Two-Sample T-Test and CI: ln Day 11, Ag (Student's t-test)

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Two-sample T for ln Day 11

Ag            N    Mean  StDev  SE Mean
coli42 (3D7)  6  10.958  0.942     0.38
Nothing       6  12.152  0.733     0.30


Difference = mu (nothing) - mu (coli42 (3D7))
Estimate for difference:  1.19420
95% lower bound for difference:  0.30095
T-Test of difference = 0 (vs >): T-Value = 2.45  P-Value = 0.018  DF = 9
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13. The results show that FMP1/AS02A induces significantly higher titers of p42-specific Ab than AS02A alone (p=0.000, T-test on natural log transformed data) – see Figure 1. The monkeys vaccinated with FMP1/AS02A showed a significantly lower parasitemia at the primary endpoint (p=0.018, independent Student-test on natural log transformed data) compared with monkeys that received AS02A – see Figure 2A and Figure 2B).

14. It is my opinion that these results show that FMP1/AS02A, a vaccine made as described at pages 52-55 in the present patent application, is protective against infection with a heterologous strain of *Plasmodium falciparum*.

15. I declare further that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and such willful false statements may jeopardize the validity of the instant patent application or any patent issuing thereon.

By: Jeffrey A Lyon Date: 31 Mar 2005